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Original Research Article

Impacts of energy feeds and supplemental protease on growth performance, nutrient digestibility, and gut health of pigs from 18 to 45 kg body weight

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ABSTRACT

A total of 144 pigs with 18.4 ± 2.3 kg initial body weight (BW) at 6 wk of age were used in a 40-d trial to evaluate effects of protease (300,000 U/kg feed, BioResource International Inc., Durham, NC, USA) on growth performance, apparent ileal digestibility (AID) of nutrients, and gut health of pigs fed diets with sorghum. Pigs were randomly allotted to 4 treatments (12 pens per treatment, 3 pigs per pen) in a 2 × 2 factorial arrangement (corn or sorghum basal diets, and 0 or 0.05% protease as 2 factors) with sex and initial BW as blocks. Experimental period had phase 1 (d 1 to 21) and phase 2 (d 22 to 40). About 65% (phase 1) and 72% (phase 2) of cereal grains were used in corn or sorghum based diets. Both grains were ground to 400 μm. Body weight and feed intake were recorded weekly. On d 35, serum was collected to quantify tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA). Titanium dioxide (0.3%) was added as an indigestible marker for an additional 4 d feeding. On d 40, 32 pigs (8 pigs per treatment) were euthanized to collect digesta from jejunum and ileum (for viscosity and AID), tissues (for morphology) and mucosa samples (for TNF-α and MDA) from duodenum, jejunum, and ileum. Replacing corn with sorghum in the diet increased ($P < 0.05$) overall average daily gain (from 756 to 787 g/day) and average daily feed intake (from 1,374 to 1,473 g/day), reduced ($P < 0.05$) overall gain:feed ratio (from 0.553 to 0.537), and did not affect AID. Pigs fed diets with sorghum had lower ($P < 0.05$) MDA content in serum (from 14.61 to 6.48 μmol/L) and jejunum (from 1.42 to 0.91 μmol/g protein), and reduced ($P < 0.05$) villus height (from 492 to 396 μm) and crypt depth (from 310 to 257 μm) in jejunum. Dietary protease improved ($P < 0.05$) AID of crude protein (from 81.8% to 86.0%), decreased MDA level (from 1.20 to 0.98 μmol/g protein) in duodenum, and increased ($P < 0.05$) the ratio of villus height to crypt depth (from 1.08 to 1.21) in duodenum. Overall, use of sorghum fully replacing corn in diets could benefit pigs with enhanced growth and feed intake potentially by reducing oxidative stress, whereas feed efficiency was compromised. Supplementation of protease improved protein digestion and maintained gut health, irrespective of sorghum or corn based diets.

1. Introduction

Sorghum can be cultivated under drier conditions when compared with maize, suggesting it could be more available to the feed manufacturers located in the dry lands. The global production of sorghum grains in 2016 was 62.64 × 10^6 t, with 12.20 × 10^6 t in the U.S. (USDA, 2017). Sorghum can substitute other cereal grains used in swine diets, showing that growth performance of pigs fed sorghum based diets may not always be comparable to that of corn based diets (Lin et al., 1987; Jondreville et al., 2001; Nyannor et al., 2007). Low-tannin sorghum was used in these experiments, indicating that tannins were not responsible for the decreased growth

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performance. Thus, kafirin might be the main factor rather than tannin, limiting the use of sorghum in non-ruminant species. Kafirin, as the prolamin storage protein in sorghum grains, has relatively low levels of basic amino acids, especially Lys (De Mesa-Stonestreet et al., 2010). Moreover, sorghum has poor protein digestibility, due to the hydrophobicity and disulfide crosslinking of kafirins (Duodu et al., 2003).

Keratinase, a class of proteolytic enzymes, has the capacity to cleave disulfide bonds, and hydrolyze soluble casein, insoluble keratin, and other proteins crosslinked by disulfide bonds (Brandelli et al., 2010). Keratinase supplementation in corn-based diets improved growth performance of pigs and poultry (Odetallah et al., 2005; Wang et al., 2011). It is hypothesized that protease supplementation with keratinase activity could have greater benefit in pigs fed sorghum based diets by hydrolyzing kafirin and thus improving protein digestibility. However, most studies of keratinase application in sorghum based diets were conducted in poultry and showed improved apparent ileal digestibility (AID) of protein and amino acid (AA) (Selle et al., 2010; Liu et al., 2013b). Additionally, the effect of sorghum based diets or protease supplementation was inconsistent in pigs (Zamora et al., 2011; Liu et al., 2013a). Therefore, the objective of this study was to determine the effect of protease on growth performance, nutrient digestibility, and gut health of pigs fed sorghum based diets at late nursery and grower stages.

2. Materials and methods

The experimental protocol was approved by North Carolina State University Animal Care and Use Committee (Raleigh, NC, USA).

2.1. Animals and experimental design

The experiment was conducted at the North Carolina Swine Evaluation Station (Clayton, NC, USA). A total of 144 barrows and gilts (18.4 ± 2.3 kg) at 6 wk of age were allotted to 4 dietary treatments in a 2 × 2 factorial arrangement (corn or sorghum basal diet, and 0 or 300,000 U keratinase/kg feed as 2 factors) based on sex and initial body weight (BW). Therefore, there were 4 dietary treatments with 12 replicate pens (6 male and 6 female pens) per treatment, with 3 pigs per pen. The experiment period was 40 d, and was divided into 2 phases: phase 1 (1 to 21 d) and phase 2 (22 to 40 d). Four diets in each phase were made separately at the North Carolina State University Feed Education Unit (Raleigh, NC, USA). Both corn and sorghum were ground to 400 μm. The analyzed nutrient value of corn and sorghum are listed in Table 1. The source of protease used in this study was Versazyme (BioResource International Inc., Durham, NC, USA). The inclusion ratio of such enzyme product was 0.05% by replacing corn or sorghum in the basal diet, so it needed to be premixed with about 5 kg ground corn or sorghum before feed mixing. The calculated values of essential nutrients in 4 experimental diets of each phase were adequate (NRC, 2012). The diet composition was summarized in Table 2. The diets were all mash feed. Pens (4.0 m × 1.4 m) with solid concrete floor were equipped with a nipple drinker and a 1-hole steel self-feeder. Pigs had free access to feed and water. Body weight and feed intake were recorded weekly. Feed efficiency was calculated as gain:feed ratio (G:F). On d 35, titanium dioxide (0.3%) was added as an indigestible marker to all diets for an additional 4 d feeding.

Table 1
Analyzed nutrient profile (%) of corn and sorghum used in the experiment (as-fed basis).

Item	Corn	Sorghum
DM	87.03	86.09
Ash	1.17	1.40
CP	7.20	9.83
NDF	8.39	8.26
ADF	2.76	4.33
Crude fat	3.43	2.61
Ca	0.01	0.05
P	0.25	0.26

DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Ca = calcium; P = phosphorus.

2.2. Sample collection

On d 35, blood samples were collected from the jugular vein with BD sterile vacutainers (Franklin Lakes, NJ, USA) for serum. Blood samples were centrifuged at 3,000 × *g* for 15 min at 4 °C to obtain the supernatant. Serum samples were stored at −80 °C until analyzed for concentrations of tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA).

On d 40, 8 pigs per treatment representing median BW of each treatment were selected and euthanized by using captive bolt. Digesta from ileum (about 20 cm before the ileal–cecal junction) was collected and stored at −20 °C for AID measurement. Mucosa sample from duodenum (2 cm after the pyloric–duodenal junction until the loop ends), jejunum (around 100 cm before the ileal–cecal junction), and ileum were stored in −80 °C for concentrations of TNF-α and MDA. Tissue sample from duodenum, jejunum, and ileum were flushed with saline solution, and stored in 10% formalin buffer at room temperature for histology evaluation.

2.3. Chemical analysis

Diets and ileal digesta were stored at −20 °C until being freeze-dried (24D × 48, Virtis, Gardiner, NY, USA). Diet and freeze-dried digesta samples were ground and analyzed for dry matter (Method 934.01, AOAC, 2006). Titanium dioxide concentration was measured at the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO, USA). Nitrogen in the feed and digesta samples was quantified using TruSpec N Nitrogen Determinator (LECO Corp., St. Joseph, MI, USA) to calculate crude protein (6.25 × N). Gross energy was determined using a calorimeter (Model 6200, Parr Instrument Company). Samples of feed and ileal digesta were analyzed sequentially for neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology Corp, Fairport, NY). Apparent ileal digestibility of dry matter (DM), gross energy (GE), crude protein (CP), NDF, and ADF were calculated using titanium concentration in the feed and digesta. The digestibility was calculated with the following equation:

$$\text{AID}\ (\%) = \left(1 - \frac{\text{Ti}_{\text{feed}} \times \text{N}_{\text{digesta}}}{\text{Ti}_{\text{digesta}} \times \text{N}_{\text{feed}}}\right) \times 100,$$

where Ti_{feed} represents the titanium concentration in the feed, $\text{Ti}_{\text{digesta}}$ is the titanium concentration in the ileal digesta, N_{feed} represents the nutrient concentration in the feed, and $\text{N}_{\text{digesta}}$ is the nutrient concentration in the ileal digesta.

2.4. ELISA measurement

Mucosa samples were homogenized (Tissuemiser, Thermo Fisher Scientific Inc., Rockford, IL, USA) on ice. The homogenate was centrifuged at 15,000 × *g* at 4 °C for 30 min to collect supernatant. The supernatant was used to determine concentrations of total protein, TNF-α, and MDA.

Table 2
Composition of corn- and sorghum-based diets.

Item	Phase 1 (d 1 to 21)		Phase 2 (d 22 to 40)	
	Corn-based diet	Sorghum-based diet	Corn-based diet	Sorghum-based diet
Ingredient, %				
Corn	65.00	0.00	72.00	0.00
Sorghum	0.00	65.00	0.00	72.00
Soybean meal	30.00	30.00	24.00	24.00
Protease[1]	0.05	0.05	0.05	0.05
L-Lys HCl	0.40	0.45	0.30	0.35
DL-Met	0.12	0.15	0.10	0.12
L-Thr	0.12	0.12	0.08	0.07
Poultry fat	1.91	1.91	1.00	1.09
NaCl	0.22	0.22	0.22	0.22
Vitamin premix[2]	0.03	0.03	0.03	0.03
Trace mineral premix[3]	0.15	0.15	0.15	0.15
$CaHPO_4$	1.00	0.90	1.07	0.92
Limestone	1.00	1.02	1.00	1.00
Total	100.00	100.00	100.00	100.00
Calculated nutrient values, %				
ME, kcal/kg	3,374	3,340	3,334	3,304
SID Lys	1.23	1.24	1.00	1.01
SID Met + Cys	0.69	0.68	0.62	0.60
SID Trp	0.21	0.21	0.18	0.18
SID Thr	0.73	0.74	0.61	0.61
Ca	0.71	0.70	0.71	0.68
STTD P	0.34	0.34	0.34	0.33
Analyzed nutrient values, %				
DM	88.86	87.77	91.82	91.82
GE, kcal/kg	–	–	3,925	3,919
CP	19.41	20.48	16.00	17.50
NDF	6.70	10.51	9.47	16.28
ADF	2.90	4.28	3.00	4.58
TiO_2[4]	–	–	0.28	0.30

ME = metabolizable energy; SID = standardized ileal digestible; STTD = standardized total tract digestibility; DM = dry matter; GE = gross energy; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] Protease was Versazyme (BRI Inc., RTP, NC, USA) at 0.05% replacing either corn or sorghum for treatment diets, providing 300,000 U keratinase activity/kg feed.
[2] The vitamin premix provided the following per kilogram of complete diet: 6,613.8 IU of vitamin A; 992.0 IU of vitamin D_3; 19.8 IU of vitamin E; 2.64 mg of vitamin K; 0.03 mg of vitamin B_{12}; 4.63 mg of riboflavin; 18.52 mg of pantothenic acid; 24.96 mg of niacin; 0.07 mg of biotin.
[3] The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.
[4] Titanium dioxide (0.3%) was added as an indigestible marker on d 35.

Total protein of serum and mucosa samples were analyzed with Pierce BCA Protein Assay Kit (23225#, Thermo Fisher Scientific Inc. Rockford, IL, USA). Concentrations of TNF-α in serum and mucosa from duodenum, jejunum, and ileum were analyzed using Porcine TNF-α Immunoassay ELISA Kit (R&D System Inc. Minneapolis, MN, USA). The detection limit range for TNF-α ELISA was 2.8 to 5.0 pg/mL. Concentrations of TNF-α in serum and mucosa samples were expressed as ng/mL and ng/mg protein, respectively. Concentrations of MDA in serum and mucosa samples from duodenum, jejunum, and ileum were analyzed using Thiobarbituric Acid Reactive Substance (TBARS) Assay Kit (Cell Biolabs, Inc. San Diego, CA, USA) following the instructions of Weaver et al. (2014). Concentrations of MDA in serum and mucosa samples were expressed as μmol/L and μmol/g protein, respectively.

2.5. Histology

Tissue samples from duodenum, jejunum, and ileum were fixed in formalin buffer and sent to North Carolina State University histology laboratory (Raleigh, NC, USA) for dehydration, embedment, and staining according to their internal standard protocol. Staining was done using hematoxylin and eosin dyes. Villus height and crypt depth were measured under an Infinity 2-2 digital CCD camera attached to an Olympus CX31 microscope (Lumenera Corporation, Ottawa, Canada). Then, the ratio of villus height to crypt depth was calculated. Lengths of 10 well-oriented intact villi and their associated crypts were measured in each slide. One person executed all the analysis of intestinal morphology.

2.6. Statistical analysis

Data were analyzed using Mixed procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The experiment was a randomized complete block design using initial BW and sex as blocking factors. The experimental unit was the pen for growth performance, while the individual pig for other measurements. Initial BW block was considered as a random effect, while ingredient (corn or sorghum), enzyme supplementation (0 or 0.05% protease), the interaction between ingredient and enzyme, and the block of sex were considered as fixed effects. Statistical differences were considered significant with $P < 0.05$. Probabilities less than 0.10 and equal or greater than 0.05 were considered as tendencies.

3. Results

3.1. Growth performance

The average initial BW of each treatment was not different from each other (Table 3). During wk 1, 3, and 5, average daily gain (ADG) was not affected by either cereal base or enzyme supplementation. During wk 4, pigs fed sorghum based diets had an increased ($P < 0.05$) ADG. So, phase 1 and overall ADG were improved ($P < 0.05$) by supplemental sorghum.

Table 3
Growth performance of pigs fed corn or sorghum based diets supplemented with and without protease.[1]

Item	Corn		Sorghum		SEM	*P*-value[2]		
	Protease, %		Protease, %			Ing	Enz	Ing × Enz
	0	0.05	0	0.05				
BW, kg								
Initial	18.36	18.42	18.43	18.44	0.67	0.608	0.726	0.808
wk 1	22.94	22.82	22.99	23.03	0.88	0.487	0.827	0.674
wk 2	27.30	26.56	27.00	27.59	1.01	0.189	0.793	0.017
wk 3	32.41	31.92	32.45	33.18	1.17	0.081	0.744	0.101
wk 4	38.11	37.89	38.80	39.54	1.35	0.015	0.590	0.308
wk 5	44.28	43.64	44.57	45.53	1.49	0.060	0.780	0.163
ADG, g								
wk 1	654	649	652	672	32	0.527	0.648	0.431
wk 2	623	535	593	651	37	0.198	0.656	0.033
wk 3	742	765	779	800	43	0.168	0.393	0.938
wk 4	815	863	907	943	43	0.006	0.159	0.840
wk 5	881	846	824	855	35	0.450	0.943	0.296
Phase 1 (wk 1 to 3)	673	650	675	707	26	0.039	0.727	0.051
Phase 2 (wk 4 to 5)	848	854	866	899	29	0.155	0.359	0.525
Overall (wk 1 to 5)	760	752	770	803	23	0.020	0.327	0.103
ADFI, g								
wk 1	1,003	1,007	1,029	1,057	50	0.073	0.427	0.557
wk 2	1,112	1,033	1,113	1,171	50	0.034	0.741	0.038
wk 3	1,346	1,343	1,381	1,525	60	0.003	0.047	0.038
wk 4	1,536	1,631	1,735	1,754	78	0.013	0.356	0.537
wk 5	1,881	1,844	1,958	2,003	81	0.040	0.938	0.462
Phase 1 (wk 1 to 3)	1,154	1,128	1,175	1,251	49	0.002	0.251	0.024
Phase 2 (wk 4 to 5)	1,708	1,737	1,846	1,879	71	0.007	0.534	0.975
Overall (wk 1 to 5)	1,375	1,372	1,443	1,502	55	0.001	0.307	0.246
G:F								
wk 1	0.655	0.642	0.635	0.637	0.009	0.164	0.577	0.414
wk 2	0.558	0.514	0.538	0.553	0.027	0.638	0.473	0.153
wk 3	0.553	0.575	0.563	0.536	0.029	0.488	0.905	0.246
wk 4	0.534	0.532	0.528	0.543	0.022	0.897	0.762	0.661
wk 5	0.472	0.461	0.421	0.433	0.014	0.008	0.999	0.414
Phase 1 (wk 1 to 3)	0.585	0.578	0.577	0.570	0.018	0.425	0.462	0.997
Phase 2 (wk 4 to 5)	0.499	0.494	0.469	0.484	0.014	0.034	0.629	0.285
Overall (wk 1 to 5)	0.555	0.551	0.535	0.539	0.009	0.029	0.993	0.571

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = feed conversion ratio.
[1] Protease was Versazyme (BRI Inc., RTP, NC, USA) at 0.05% replacing either corn or sorghum for treatment diets, providing 300,000 U keratinase activity/kg feed.
[2] Ing: main effect of ingredient; Enz: main effect of protease; Ing × Enz: interaction effect between ingredient and protease.

During wk 1, average daily feed intake (ADFI) tended to be increased ($P = 0.073$) by supplemental sorghum. From wk 2 to 5, ADFI was significantly increased ($P < 0.05$) by supplemental sorghum. Regardless of the diet type, dietary protease improved ($P < 0.05$) ADFI during wk 3.

From wk 1 to 4, G:F was not affected by diet type or protease supplementation, but was greatly reduced ($P < 0.05$) by supplemental sorghum in wk 5 (from 0.467 to 0.427), which resulted in a lower G:F in phase 2 and the overall period when pigs were fed sorghum based diet.

3.2. Apparent ileal digestibility

Apparent ileal digestibility of nutrients was not influenced by completely replacing corn with sorghum (Table 4). However, supplementation of protease tended to increase ($P < 0.10$) AID of DM, GE, and NDF, and improved ($P < 0.05$) AID of CP. There were no interactions observed.

3.3. Tumor necrosis factor-α and malondialdehyde

Concentrations of TNF-α in serum or mucosa samples were not affected by cereal base or supplementing protease (Table 5). Pigs fed sorghum basal diets had lower ($P < 0.05$) MDA content in serum and jejunum mucosa. Malondialdehyde level in duodenum mucosa was reduced ($P < 0.05$) by supplementation of protease. No interactions were observed.

Table 4
Apparent ileal digestibility (AID, %) of DM, CP, GE, NDF, and ADF in pigs fed corn or sorghum based diets supplemented with and without protease.[1]

Item	Corn		Sorghum		SEM	*P*-value[2]		
	Protease, %		Protease, %			Ing	Enz	Ing × Enz
	0	0.05	0	0.05				
DM	82.1	84.9	82.5	83.9	1.3	0.966	0.078	0.462
CP	81.7	85.7	81.8	86.2	1.7	0.868	0.014	0.898
GE	84.5	87.2	85.5	86.8	1.1	0.784	0.063	0.532
NDF	41.9	44.8	42.6	43.7	1.1	0.838	0.071	0.405
ADF	31.5	33.3	30.3	31.7	1.5	0.345	0.299	0.877

DM = dry matter; CP = crude protein; GE = gross energy; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] Protease was Versazyme (BRI Inc., RTP, NC, USA) at 0.05% replacing either corn or sorghum for treatment diets, providing 300,000 U keratinase activity/kg feed.
[2] Ing: main effect of ingredient; Enz: main effect of protease; Ing × Enz: interaction effect between ingredient and protease.

3.4. Histology

Dietary protease increased ($P < 0.05$) the ratio of villus height to crypt depth in duodenum. Pigs fed sorghum based diets had lower ($P < 0.05$) villus height and crypt depth in jejunum (Table 6).

Table 5
Tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA) in serum and mucosa samples of pigs fed corn or sorghum based diets supplemented with and without protease.[1]

Item	Corn		Sorghum		SEM	*P*-value[2]		
	Protease, %		Protease, %			Ing	Enz	Ing × Enz
	0	0.05	0	0.05				
TNF-α								
Serum, pg/mL	91.46	80.33	88.71	84.74	12.86	0.949	0.562	0.783
Duodenum, pg/mg protein	8.92	9.51	8.34	8.86	0.69	0.379	0.429	0.956
Jejunum, pg/mg protein	7.03	6.19	7.01	5.71	0.64	0.454	0.208	0.469
Ileum, pg/mg protein	5.61	5.77	5.55	4.59	0.42	0.203	0.355	0.160
MDA								
Serum, μmol/L	15.62	13.59	6.73	6.23	1.83	0.001	0.496	0.680
Duodenum, μmol/g protein	1.20	0.99	1.19	0.97	0.06	0.855	0.023	0.985
Jejunum, μmol/g protein	1.40	1.43	1.00	0.81	0.12	0.003	0.496	0.372
Ileum, μmol/g protein	0.79	0.75	0.72	0.79	0.07	0.844	0.760	0.488

[1] Protease was Versazyme (BRI Inc., RTP, NC, USA) at 0.05% replacing either corn or sorghum for treatment diets, providing 300,000 U keratinase activity/kg feed.
[2] Ing: main effect of ingredient; Enz: main effect of protease; Ing × Enz: interaction effect between ingredient and protease.

4. Discussion

4.1. Sorghum

In the current study, complete replacement of corn with sorghum increased ADG and ADFI, whereas resulting in a reduced feed efficiency in pigs due to increase in ADFI. In other studies, finisher pigs were fed sorghum based diets and pigs tended to have increased ADFI and ADG, but unaffected G:F (Paulk et al., 2015), whereas Kim et al. (1998) observed that pigs fed sorghum based diets had unchanged feed intake and daily gain, but a lower feed efficiency. Healy et al. (1994) reported that nursery pigs fed diets with sorghum had reduced ADG, ADFI, and feed efficiency compared with pigs fed corn based diets. Age of pigs could cause different growth responses to sorghum. In addition, source of sorghum related to tannin content could also cause different growth responses of pigs but it was not clear to identify the source of sorghum or difference in tannin contents in sorghum among studies. In the current study, an increase in ADG by the use of sorghum is a clear benefit whereas a reduction in feed efficiency is not. Considering that the pigs used in this study included late nursery stage (from 18 kg body weight), benefits of using sorghum obtained from enhanced ADG may be greater than the loss from reduced feed efficiency.

Sorghum diet contains less digestible protein (Mariscal-Landín et al., 2010), but we did not observe reduced AID of nutrients caused by replacing corn with sorghum in the diets. Such response can be attributed to the particle size of cereal grains. Previous research concluded that feed efficiency and nutrient digestibility of pigs can be improved by reducing the particle size of sorghum (Healy et al., 1994; Paulk et al., 2015). At the particle size of 600 um, AID of AA were lower in growing pigs fed sorghum when compared with those fed corn based diets (Pedersen et al., 2007). So, reducing particle size of grains to 400 μm in this study could improve nutrient ileal digestibility in both corn and sorghum based diets, and such improvement eliminated the possible differences caused by diet type. However, unaffected AID did not result in the same feed efficiency in this study. This could attribute to the differences in contribution from hindgut fermentation and differences in endogenous loss occurred in the small intestine, both of which could be affected by diet types (Morales et al., 2002; Stein et al., 2007).

Besides growth performance and nutrient digestibility, gut health is another criteria important in pig production. Gut tissues receive continuous immune challenges and oxidative stress. Ethanolic extract of black sorghum bran significantly hindered the production of the pro-inflammatory cytokines TNF-α (Burdette et al., 2007). However, this effect was not observed in non-tannin sorghum (Moraes et al., 2012). Sorghum used in the current study was low tannin cultivar. This corresponded with our finding that sorghum diets had no influence on inflammatory status in the intestine of pigs. Taylor et al. (2014) demonstrated that methanol extract of sorghum has antioxidant capacities. Anti-oxidant activities of sorghum correlated positively with levels of total phenolic acids (Awika and Rooney, 2004; Burdette et al., 2007). Oxidative stress is caused by production of oxidants, and would leads to oxidative damage to the cell components, such as the oxidation of proteins and lipids in the cell (Schieber and Chandel, 2014). Liu et al. (2000) reviewed that the level of oxidative stress can be evaluated by the content of lipid peroxidation, expressed as MDA concentrations which was also tested in our previous studies (Berchieri-Ronchi et al., 2011; Zhao et al., 2013; Shen et al., 2014; Sun et al., 2015). In our study, replacing corn with sorghum decreased MDA levels in serum and jejunum. Reduction in MDA partly supports a reduction in oxidative stress. Therefore, sorghum utilized in the diets exerted a positive effect on maintaining gut health by potentially reducing oxidative stress. Yuan et al. (2007) showed that reduced oxidative stress is associated with improved growth performance in pigs.

Table 6
Villus height (H), crypt depth (D) and the ratio of villus height to crypt depth (H:D) of duodenum, jejunum and ileum in pigs fed corn or sorghum based diets supplemented with and without protease.[1]

Item	Corn		Sorghum		SEM	*P*-value[2]		
	Protease, %		Protease, %			Ing	Enz	Ing × Enz
	0	0.05	0	0.05				
Duodenum								
H, μm	473	557	499	495	25	0.478	0.128	0.095
D, μm	437	443	474	431	14	0.402	0.201	0.096
H:D ratio	1.09	1.27	1.06	1.15	0.06	0.246	0.038	0.470
Jejunum								
H, μm	497	486	389	403	24	0.004	0.963	0.611
D, μm	322	297	248	266	15	0.002	0.818	0.158
H:D ratio	1.57	1.64	1.58	1.52	0.08	0.479	0.940	0.411
Ileum								
H, μm	423	409	404	434	23	0.836	0.682	0.310
D, μm	234	235	254	235	12	0.409	0.468	0.430
H:D ratio	1.86[b]	1.73[a]	1.60[a]	1.86[b]	0.09	0.484	0.476	0.044

[ab] Mean lacking a common superscript differ ($P < 0.05$).
[1] Protease was Versazyme (BRI Inc., RTP, NC, USA) at 0.05% replacing either corn or sorghum for treatment diets, providing 300,000 U keratinase activity/kg feed.
[2] Ing: main effect of ingredient; Enz: main effect of protease; Ing × Enz: interaction effect between ingredient and protease.

In this study, villus height and crypt depth of jejunum were decreased by supplemental sorghum. The change in gut morphology of pigs may be associated with the lower feed efficiency. As one of the anti-nutritional factors, tannin might be responsible for the changes in the gut morphology and function in pigs (Pluske et al., 1997). Tannin supplementation (up to 4.5 g/kg feed per day) reduced crypt depth in the ileum of weaned pigs (Biagi et al., 2010). However, we utilized the low-tannin cultivar in this study. There might be a range of tannin in the diet that is not sufficient to affect immune status, but still could change gut morphology of pigs, which needs further research.

4.2. Protease

The protease supplemented in this study is produced by *Bacillus licheniformis* PWD-I, and has the ability of degrading keratins and a wide range of other proteins (Wang et al., 2006). It is well recognized for attacking highly cross-linked and recalcitrant structural proteins, and used as a feed enzyme to improve nutritional value of proteins existing in the diet (Gupta et al., 2013). In our study, dietary supplementation of protease improved ADFI only during the third week, but did not significantly affect ADG and G:F. It was suggested that endogenous protease supplementation could increase starch and protein digestibility without improving growth performance (Liu et al., 2013a). Dietary protease increased AID of CP in both corn and sorghum based diets in the present study. Similarly, AID of CP and most AA in growing pigs were improved by adding protease to corn based diets (Wang et al., 2011). The effect of protease in corn-soybean meal (SBM) diets might be attributed to the hydrolysis of cystine disulfide bonds found in soybean proteins, such as glycinin and β-conglycinin, and thus improved protein digestion (Hou and Chang, 2004). Studies in poultry showed that adding protease to sorghum based diets improved amino acid and protein digestibility in chickens (Selle et al., 2010; Liu et al., 2013b), which might be due to similar mechanism that protease hydrolyzes the less digestible proteins such as kafirin, to make them more available to animals. However, such improvement did not result in increased feed efficiency in this study. As our aforementioned discussion, the inconsistency between AID and feed efficiency could be due to the energy produced via fermentation, which might compensate for the losses.

Dietary factors in the lumen will lead to relatively quick changes in the mucosa due to the interaction between the mucosal surface and the intestinal digesta, and such changes could affect gut health, nutrient digestibility, as well as growth performance. In our study, dietary protease did not affect the levels of TNF-α in serum and mucosa samples. However, reduced TNF-α levels were observed in serum, duodenum, and jejunum of pigs fed diets with protease (Guo et al., 2014; Park et al., 2015). Interestingly, dietary protease reduced MDA level in duodenum in our study. Guo et al. (2014) also reported adding protease decreased MDA level in serum of nursery pigs fed corn and 30% SBM based diets. In broilers, supplementing protease decreased MDA level in serum and ileum of birds fed with corn, SBM, and distillers dried grains with soluble based diets. As an indicator of lipid peroxidation levels, MDA also represents levels of reactive oxygen species, indicating the oxidative stress in the tissue of animals, and influenced the morphology and cell proliferation. The reduced MDA level in duodenum corresponded with an improved ratio of villus height to crypt depth in the duodenum. An increase in the ratio of villus height to crypt depth was associated with better nutrient absorption, better gastrointestinal health, and improved growth performance (Wang et al., 2008). Similar improvement in morphology of small intestine was also observed by Guo et al. (2014) and Park et al. (2015). The hydrolysis of dietary protein might contribute to such improvement.

5. Conclusion

Overall, completely replacing corn with sorghum was not a big concern to pigs in late nursery and grower stages. On the contrary, sorghum based diets might be potentially beneficial due to the increased feed intake and weight gain whereas potentially reducing oxidative stress. However, reduction in feed efficiency due to increased feed intake by the use of sorghum should not be ignored. Supplementation of protease improved protein digestion and possibly maintained gut health condition supported by potential reduction in oxidative stress and enhanced morphology, irrespective of sorghum or corn based diets.

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